RMIT UNIVERSITY

Poultry trial

Efficacy and safety of BDM-I as a feed additive for performance enhancement in commercial broiler chickens.

Interim Report
22 June 2004

Location of research

Field trial: Longerenong Agricultural College
Institute of Land and Food Resources
University of Melbourne
Horsham Vic 3401

Laboratory analyses: Biotechnology & Environmental Biology
School of Applied Sciences
RMIT University
Plenty Road
Bundoora Vic 3083

Names and affiliations of Investigators

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Start/finish date of field trial

4 May 2004 to 7 June 2004

Chief Investigator
Gina Nicoletti

Poultry Trial 2

Interim report

Summary of results

An earlier pilot field trial with 540 unsexed commercial broiler chicks, undertaken at Longerenong College poultry facility from September 30 to November 20, 2003, indicated that birds (180 per treatment group with 3 replicates) fed additive free commercial diets containing either BDM-I in canola oil (25 mg/kg feed), Zinc Bacitracin (25 mg/kg feed) or no additive showed no significant difference in weight gain or feed conversion at 6 weeks.
Because of the failure of positive control or test treatment to show performance enhancement it was decided to repeat the trial using a higher concentration of BDM-I (50 mg/kg feed in water/Tween 20), more replicates and more stressful poultry rearing conditions (old litter and a denser stocking rate). A second untreated control group with new litter was added to isolate any effect from litter quality.

This trial has confirmed no significant difference in weight gain at 5 weeks in chicks fed BDM-I (50 mg/kg) or Zn Bacitracin (25 mg/kg) supplemented diets compared to chickens on untreated feed.
BDM-I demonstrated a significant 9.2% reduction ($P = 0.003$) in mean feed intake compared to untreated control birds while Zn Bacitracin showed a 2.9% reduction ($P = 0.2$). BDM-I was over 200% better than Zn Bacitracin in reducing feed intake ($P = 0.02$).

BDM-I also showed a significantly improved feed conversion ratio with a 6.8% reduction ($P = 0.008$) over untreated feed compared to a 4% reduction by Zn Bacitracin ($P = 0.08$). Mortality for both treatments was 2.5% compared to 5.1% for control birds but the reduction was not statistically significant.
The use of old or new litter had no effect on weight gain, feed intake or feed conversion.
Monitoring by cloacal swab showed variable and low colonisation rates for *Salmonella* and *Campylobacter*.
Birds on all treatments were healthy and showed normal behaviour and appearance.

Trial protocol

The trial was conducted at Longerenong Agricultural College in the same poultry facility as the first trial over 34 days from 4 May 2004 to June 7 2004. The trial was terminated at 34 days instead of the planned 42 days because of circumstances extraneous to the trial design or conditions of conduct or results of the trial. From 34 days all birds were fed an untreated diet to 38 days and slaughtered.

Three groups of 288 one-year old un-sexed commercial broiler chicks were subjected to 3 feed treatments (4 replicates per treatment).

1 Negative control: untreated feed
2 Positive control: Zn bacitracin 25 mg/kg (Albac 150)
3 Test compound: BDM-I 50 mg/kg (Water/Tween 20)

Treatments were added to a commercial range of Free Range Broiler feeds free of additives or coccidiostat.
Treatments were randomly allocated to 12 pens (4 replicates of 72 birds) at a stocking density of 0.5 sq ft/bird using old litter from another poultry rearing shed at the Longerenong poultry facility.
Birds were monitored daily for appearance, behaviour, general health status, morbidity and mortality and for:

1. weight gain at 0, 7,14, 21, 28 and 34 days
2. feed consumption and feed conversion to 7, 14, 21, 28 and 34 days
3. colonization with *Campylobacter* and *Salmonella* by standard microbiological analysis of cloacal swab
4. BDM-I residues in blood and tissues at 34 days
5. appearance, behaviour, general health and well-being, daily
6. morbidity and mortality, daily

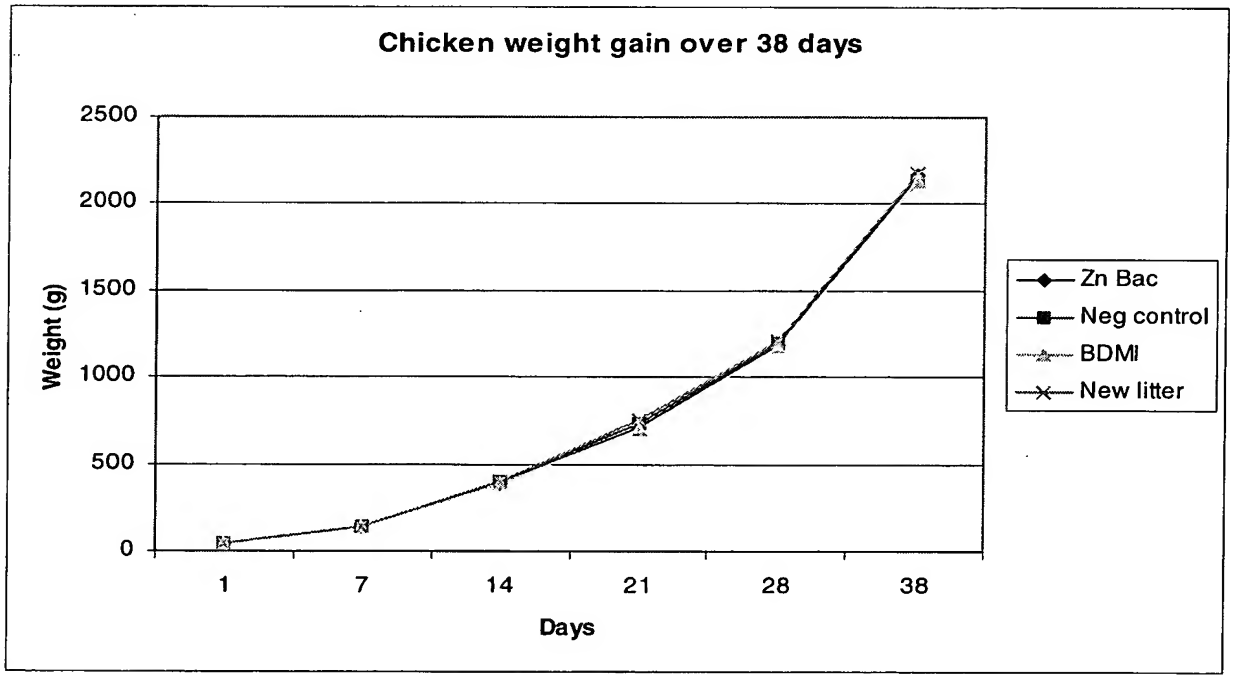
Results

Weight gain

Under the conditions of rearing at the Longerenong facility there was no significant difference in weight gain at 38 days for chickens given feed treated with BDM-I 50 mg/kg feed or Zn Bacitracin 25 mg/kg feed compared to chickens receiving untreated feed (Table 1).

Table 1. Mean body weights (g) at 38 days of chickens fed a normal diet and a diet supplemented with BDM-I (50 mg/kg) or Zn Bacitracin (25 mg/kg).

	Mean body weight (g)				
	Replicate				Mean ± SE
Treatment	1	2	3	4	
Zn Bacitracin	2142	2161	2121	2224	2162 ± 22
Neg control	2120	2174	2143	2114	2137 ± 13
BDMI	2151	2163	2095	2116	2131 ± 31
Neg control New litter	2289	2174	-	-	2231 ± 57



Feed intake and feed conversion ratio

The mean feed intake/bird for BDM-I treated birds at 34 days was 9.2% lower than that of untreated controls compared to a 2.9% reduction for Zn Bacitracin treated birds. The lowered feed intake with BDM-I treated feed was statistically significant (P = 0.003) while that of Zn Bacitracin was not significant (P = 0.2). The reduction in feed intake for BDM-I was also 6.5% lower than that for Zn Bac (P=0.02) (Figure 1).
BDM-I significantly improved the feed conversion ratio by 6.8% (P = 0.008) over untreated feed compared to a 4% reduction by Zn Bacitracin (P = 0.08) (Figure 2).

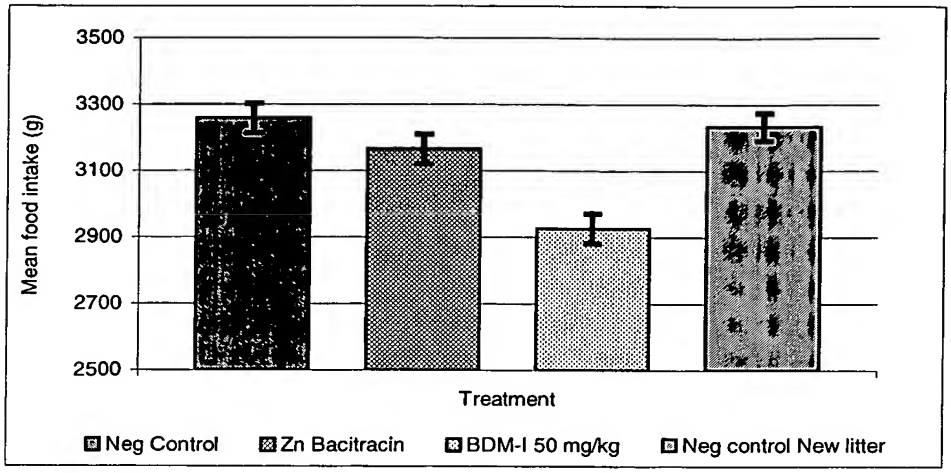


Figure 1 Mean feed intake (g) at 34 days in chickens fed a normal diet and a diet supplemented with BDM-I (50 mg/kg) or Zn Bacitracin (25 mg/kg). Percent reduction in feed intake by BDM-I compared to negative control was 9.2% (P = 0.003 two tailed t-test equal variance) and for Zn Bacitracin was 2.9% (P = 0.2). BDM-I was 6.5% better than Zn Bacitracin in reducing feed intake (P = 0.02).

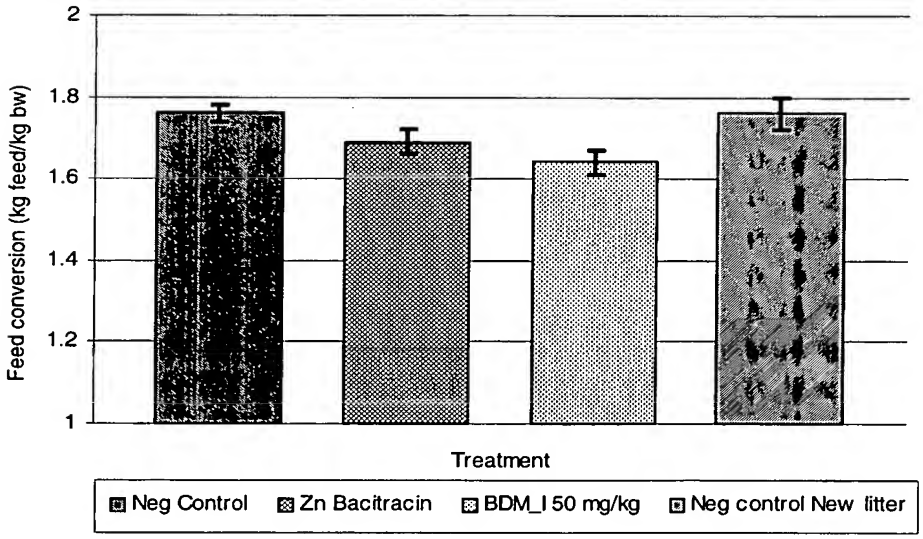


Figure 2 Feed conversion (kg feed/kg bird weight) at 34 days days in chickens fed a normal diet and a diet supplemented with BDM-I (50 mg/kg feed) or Zn Bacitracin (25 mg/kg). BDM-I significantly reduced the feed conversion ratio by 6.8% (P = 0.008 Two-tailed t-test equal variance) compared to untreated feed. Reduction by Zn Bacitracin was 4 % (P = 0.08).

New litter vs old litter

There was no difference in weight gain, feed intake or feed conversion ratio at 34 days for birds receiving untreated feed placed on new litter at the start of the trial compared to those placed on old litter from another poultry rearing shed at the start of the trial. Litter was not changed for trial duration for any treatment group.

Mortality

Both BDM-I and Zn Bacitracin reduced mortality by 50% compared to untreated controls, although the reduction was not statistically significant (ANOVA).

Treatment	Total No. birds	Deaths	%mortality
Negative control	288	14	5.1
Zn Bacitracin 25 mg/kg	290	7	2.5
BDM-I 50 mg/kg	288	7	2.5

Eimeria Trial MC04/27
Anti-coccidial efficacy of BDM-I

Analysis of results

The raw data presented in the report has been reconfigured into the following tables and figures for consideration.

Effect on oocyst excretion

There is a dose response for BDM-I at 5 days but not at 6 and 7 days (Table 1 & Figure 1).
After 5 days treatment the reduction in OPG count for 5, 50 and 250 mg/kg oral was respectively 78%, 88% and 99%.
BDM-I in feed, with 57% reduction, was least effective in lowering counts.
The data is heavily skewed as can be seen from the quartile counts and the difference between the mean and median counts.

Effect on bird weight

Bird weights after 12 days of treatment are shown in Figure 2 and % change in weight in Table 2.
There is a 25% reduction in weight gain for treatment with toltrazuril (P = 0.001 2-tail t-test) and 28% for BDM-I 250 mg/kg in feed (P=0.004). We also noted weight loss at 200 and 400 mg/kg bw presented in feed in the 28 day cumulative toxicity trial.
The weight changes for oral dosing of BDM-I, while indicative of a dose response, are not statistically significant.
The difference between BDM-I oral dosing and feed impregnation is unexplained.
(Perhaps it is a pity we cannot deliver BDM-I in water?)

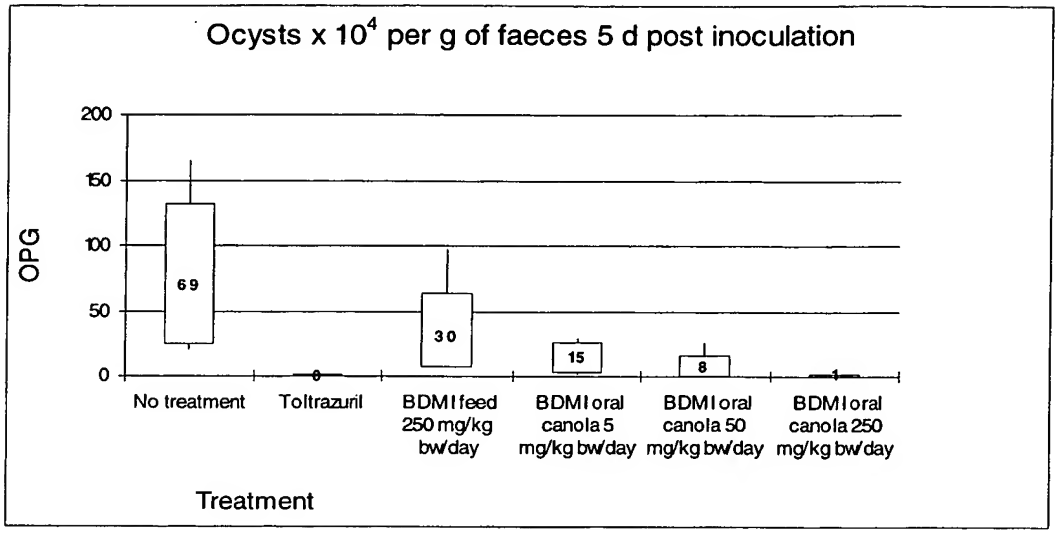
Histology

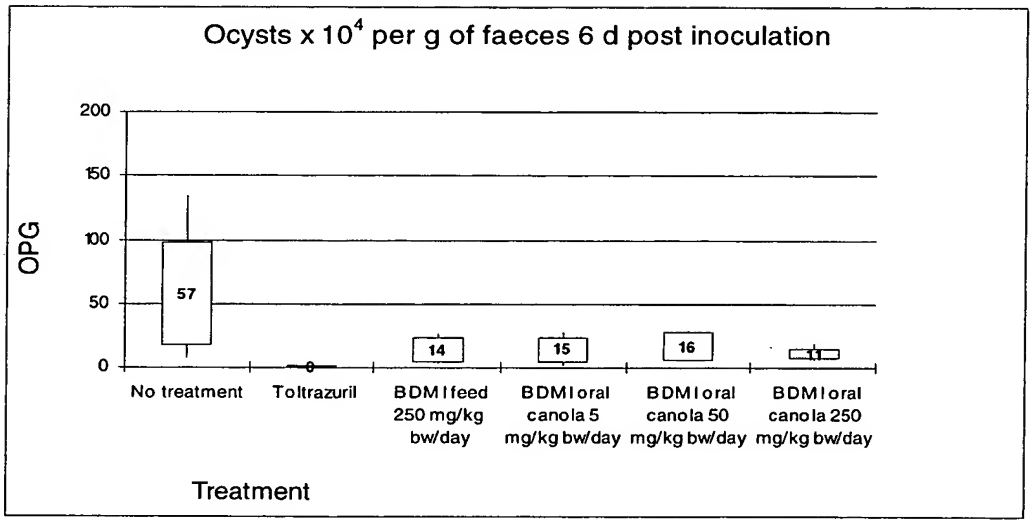
Histology results are given only for 5-day post challenge
Histological effect is presented as a score per group of 5 birds (Table 3).
Not sure how to correlate OPG counts with histology. One set of OPG counts for 5 birds at 5, 6 and 7 days are included. Grant suggests comparison of histology with OPG since birds can be individually identified and related to counts.
Oocyst output is ranked by OPG/inoculation dose and is also categorised as low medium and high. ?Correlation between category and oocyst count. The histology scores for *E. necatrix* and *E. tenella* appear to correlate with total OPG/g faeces for all 4 strains.
Comparing scores with control scores for each species, can one infer that the inhibitory effect of BDM-I is greater on *E. tenella* than on *E. necatrix*? If we compare the oocyst counts per dose quoted by Grant, BDM-I has an effect on the strains with high output counts (E. tenella, 150 oocysts/dose and E. necatrix 100 oocysts/dose)
Does the histology suggests good activity only against one of 4 species? Is this a drawback for a coccidiostat?

Table 1 Oocysts/g faeces x10,000 at 5, 6 and 7 days post inoculation with 4 vaccine strains of *Eimeria* (*E. acervulina, E. maxima, E. tenella, E, necatrix*)

Day 5	Mean	max	min	First quartile	3rd quartile
No treatment	69	165	21	24	133
Toltrazuril	<LD[1]				
BDMI feed 250 mg/kg bw/day	30	98	7	8	63
BDMI oral canola 5 mg/kg bw/day	15	29	1	3	26
BDMI oral canola 50 mg/kg bw/day	8	25	0	0	16
BDMI oral canola 250 mg/kg bw/day	1	3	0	0	2
Day 6					
No treatment	57	134	8	18	97
Toltrazuril	<LD[1]				
BDMI feed 250 mg/kg bw/day	14	26	4	4	23
BDMI oral canola 5 mg/kg bw/day	15	28	1	5	23
BDMI oral canola 50 mg/kg bw/day	16	30	5	6	28
BDMI oral canola 250 mg/kg bw/day	11	18	6	7	15
Day 7					
No treatment	109	201	7	10	183
Toltrazuril	<LD[1]				
BDMI feed 250 mg/kg bw/day	2	4	1	1	3
BDMI oral dose in canola oil 5 mg/kg bw/day	16	29	5	8	26
BDMI oral canola 50 mg/kg bw/day	13	18	9	9	18
BDMI oral canola 250 mg/kg bw/day	11	22	5	7	16

1 LD = less than whatever level of detection applies to the method





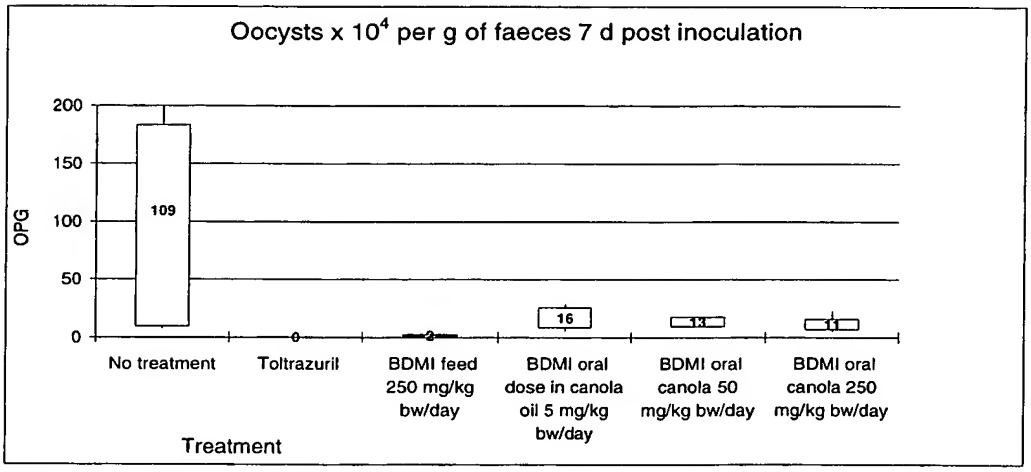


Figure 1 Oocysts x 10^4/g faeces at 5, 6 and 7 days post inoculation of 5 day-old broiler chicks with ~4000 oocysts of Eimeria species (x4)

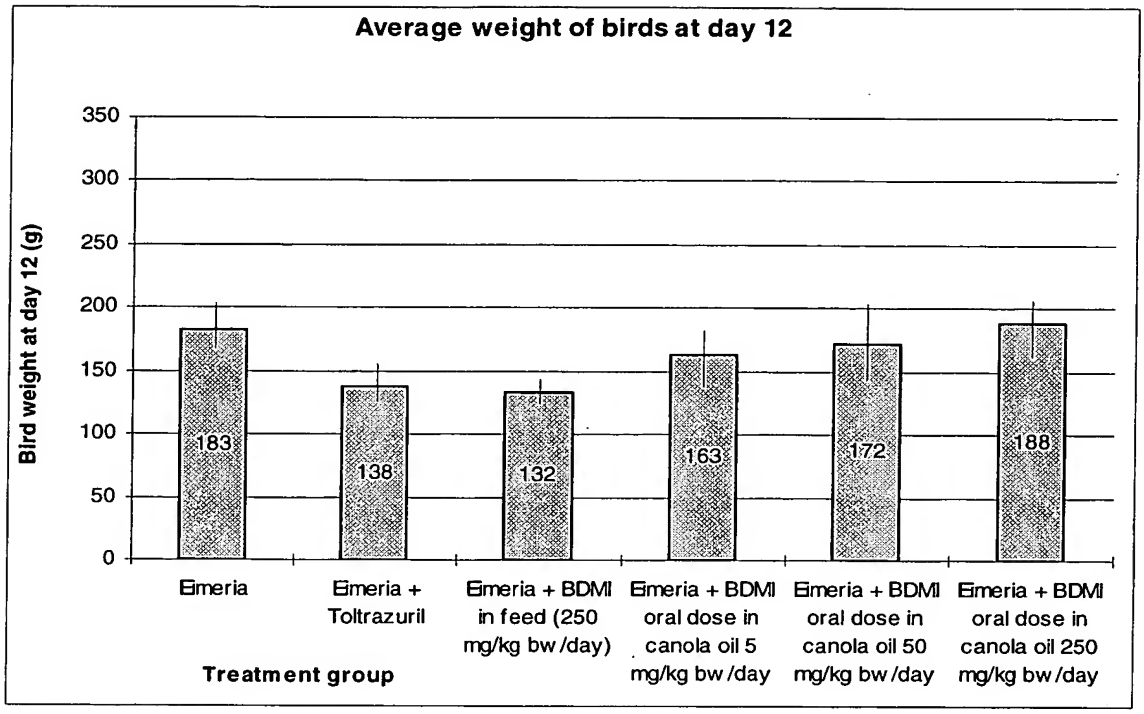


Figure 2 Mean weight of birds (5) after 12 days treatment. Treatments with toltrazuril and BDM-I in feed show significant weight reduction (2-tailed t-test)

Table 2 Percent weight change after 12 days treatment

	Weight (g)	% weight change from normal
No treatment	183	
Toltrazuril	138	-25
BDMI in feed (250 mg/kg bw/day)	132	-28
BDMI oral canola oil 5 mg/kg bw/day	163	-11
BDMI oral canola oil 50 mg/kg bw/day	172	-6
BDMI oral canola oil 250 mg/kg bw/day	188	+3

Table 3 Histology group score (5 birds) 5 days post challenge of 5 day-old broiler chicks with ~4000 oocysts of Eimeria species (x4)

	E.acervulina	*E. maxima*	*E. necatrix*	*E tenella*	OPG/g faeces
Eimeria	5	5	3	4	69
Toltrazuril	0	0	0	0	<LD
+ BDMI in feed (250 mg/kg bw/day)	5	4	3	4	30
+ BDMI oral canola oil 5 mg/kg bw/day	3	5	2	1	15
+ BDMI oral canola oil 50 mg/kg bw/day	5	5	2	1	8
+ BDMI oral canola oil 250 mg/kg bw/day	3	4	0	0	1
Oocyst output/dose	50	100	100	150	

	Q1	Mean	max	min	Q3	Median
No treatment	18	57	134	8	97	54
Toltrazuril	0	0	0	0	0	0
BDMI feed 250 mg/kg bw/day	4	14	26	4	23	14
BDMI oral canola 5 mg/kg bw/day	5	15	28	1	23	17
BDMI oral canola 50 mg/kg bw/day	6	16	30	5	28	14
BDMI oral canola 250 mg/kg bw/day	7	11	18	6	15	10